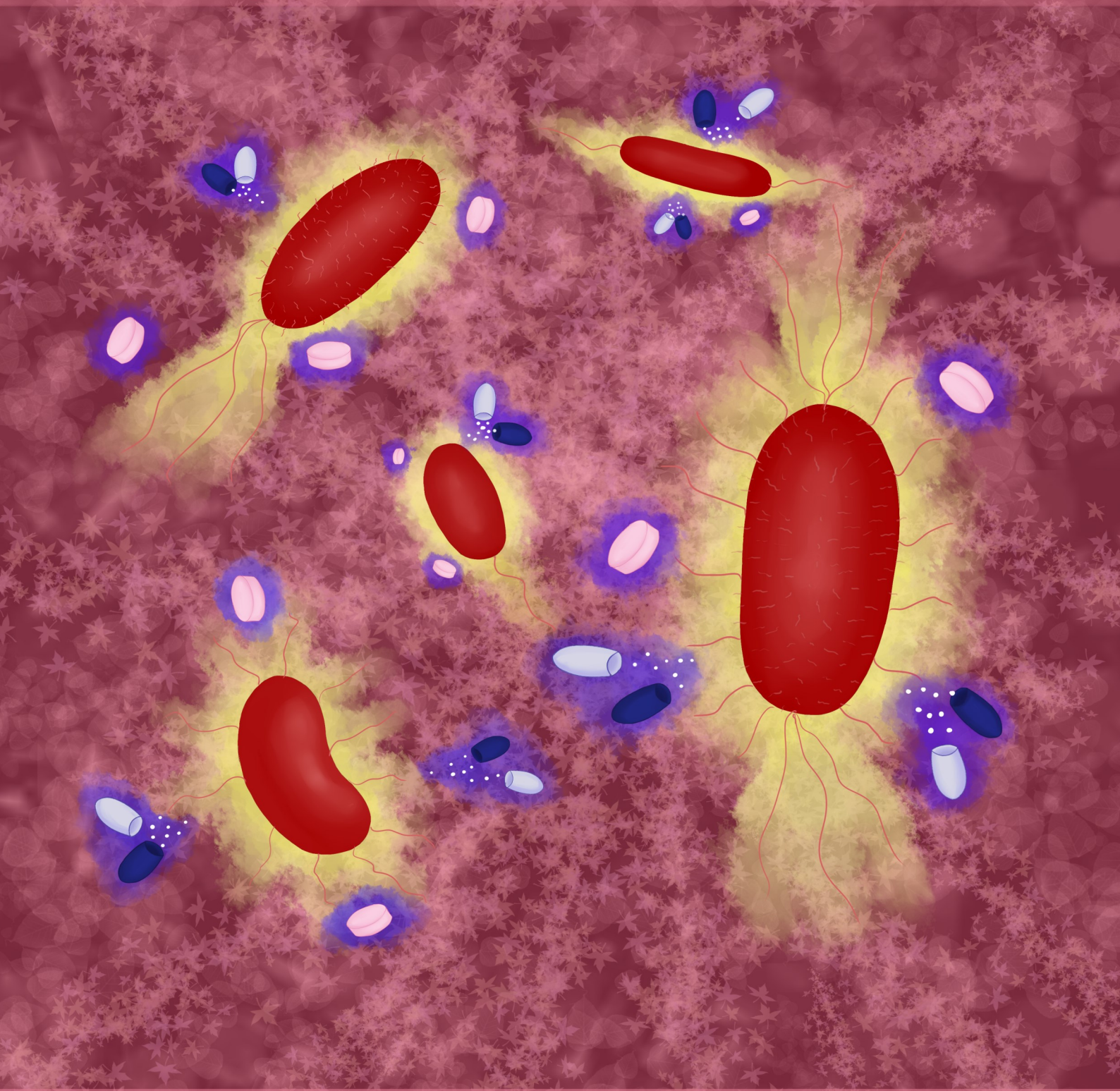
Drugs and Beyond

Fighting Bacterial Resistance



Insight into the research of the Nobel Prize Winner Ben Feringa

Discovery of a receptor facilitating the invasion of Salmonella

First Dutch researcher with the Bjorn Ekwall Memorial award

Dear HPPS community,

Welcome to this issue of Drugs and Beyond! We have been working on this issue for the past semester to provide you with new updates about the HPPS community, such as running and recruiting projects and internship abstracts. The focus of this issue is antibiotics and the problems they entail. We will take you on a journey from the discovery of the first antibiotics, their working mechanisms, and delve into antibiotic resistance. Next to plenty of interesting information about antibiotics, we provide you with the usual contents, such as several internship abstracts from CPS students, exciting news about the HPPS community and the university, PhD theses, and more details on several awareness days. This is also the last issue some of our members worked on, as they are graduating and finishing up HPPS. We thank everyone for their hard work and wish them all the best for their future!

We hope you will enjoy this Drugs and Beyond issue before the summer holiday, and wish you all a relaxing break!

We are also looking forward to seeing you again for our next issue, which will be ready before the Christmas break!

The Drugs and Beyond team

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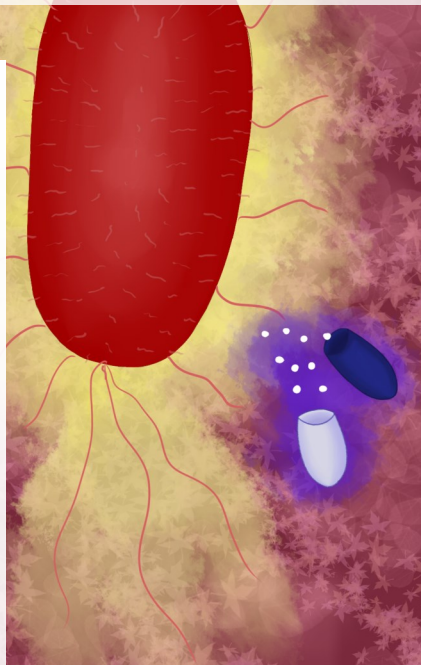
Table of Content

Letter to HPPS Community	1
Mechanisms of antibiotic action and classification	2
Production of antibiotics	3
Rise in antibiotic resistance	4
New and upcoming antibiotics	6
Nobel Prize Winner Ben Feringa	8
Gut microbiota	9
Green Chemistry project abstract	11
Internship abstracts	12
PhD abstracts	14
Latest news	15
Awareness calendar	16
References	19
Next time…	21



Mechanisms of antibiotic action and classification

Antibiotics are natural or synthetic compounds that interfere with bacteria by either inhibiting their growth or actively killing them. This gives rise to the first distinction in antibiotic classification: bacteriostatic and bactericidal antibiotics. Bacteriostatic antibiotics inhibit reproduction of bacteria, but do not actively kill them. Conversely, bactericidal antibiotics actively kill bacteria. (1) Although this distinction seems very clear in a laboratory setting, the effect of antibiotics in a clinical setting highly depends on drug concentration and the bacterial species that is targeted. (2) Certain antibiotics may act as bacteriostatics on one type of bacteria, while actively killing another. Besides these two classification categories, antibiotics are categorized based on their mechanisms. Three major mechanisms can be discriminated: inhibition of cell wall synthesis or maintenance, inhibition of nucleic acid synthesis, and inhibition of protein synthesis (see **Figure 1**).

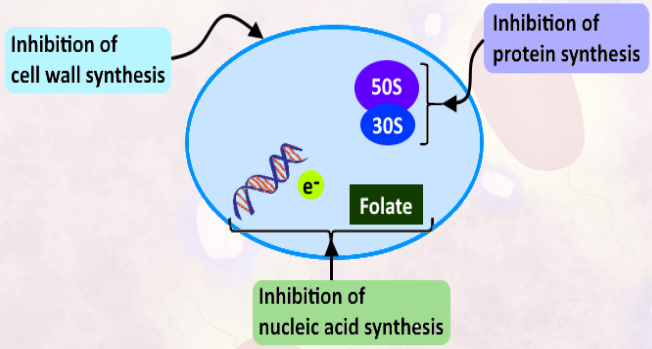


Figure 1. The three major mechanisms by which antibodies work.

Antibiotics that interfere with the cell wall and their mechanisms were elaborately described by Labert et al. (2002) in "Molecular Medical Microbiology", chapter 27. (3) In short, this antibiotic class includes four subtypes: beta lactams, vancomycin, daptomycin, and polypeptides. Examples of the latter class are bacitracin or colistin. Beta lactam antibiotics are generally bactericidal, and are the most commonly used class of antibiotics. They inhibit the enzyme DD-transpeptidase in the final step of peptidoglycan synthesis. This peptidoglycan is essential in the formation of bacterial cell walls, and binding of the DD-transpeptidases to beta lactams prevents proper formation of the cell wall. In the class of beta lactams, we find penicillins, cephalosporins, carbapenems (e.g. meropenem and ertapenem), and monobactams (e.g. aztreonam). Penicillins can be divided into four subtypes: natural penicillins (e.g. penicillin G), aminopenicillins (e.g. amoxicillin), Anti-staphylococcal penicillins (e.g. nafcillin) , and Anti-pseudomonal penicillins (e.g. piperacillin). Cephalosporins consist of generations 1 to 5, including cefazolin, cefotetan, ceftriaxone and defpodoxime, defepime, and ceftaroline, subsequently.

Antibiotics that affect nucleic acid synthesis can be further divided into three mechanisms of action. Firstly, they can convert the type II topoisomerases DNA gyrase and Topoisomerase IV. These enzymes are involved in DNA splicing for supercoiling, and can be converted into toxic enzymes that fragment the chromosome, which then leads to cell death. These antibiotics are called quinolones. (4) Secondly, they can inhibit folate (one of the B vitamins) synthesis. An example of an antibiotic treatment that does this is trimethoprim-sulfamethoxazole. Through the inhibition of folate, they disrupt proper DNA and RNA synthesis. (5) Lastly, antibiotics such as metronidazoles and nitrofurans, can create free radicals that interact with DNA, causing DNA strand breakage. (6)

The third main mechanism is the disruption of protein synthesis by targeting ribosomal subunits. They either affect formation or functioning of the 50S subunit or the 30S subunit. Antibiotics that target the 50S subunit include macrolides, clindamycin, linezolid, streptogramins, and chloramphenicol, and antibiotics that work on the 30S subunit include aminoglycosides, tetracyclines, and tigecycline. (7) An overview of possible mechanisms by which these antibiotics act is given in **Figure 2**.

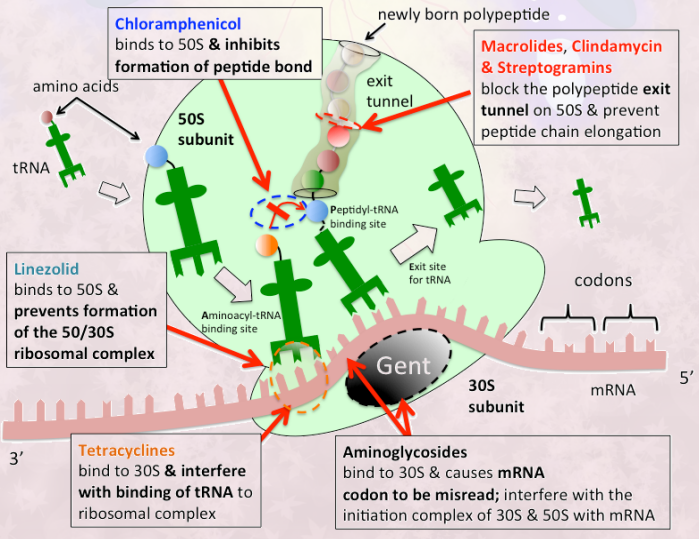


Figure 2. Examples of antibiotics that interfere with protein synthesis by targeting the ribosome and their sites of action. [Source: Tulane University, School of Medicine: Protein Synthesis Inhibitors, http://tmedweb.tulane.edu/pharmwiki/doku.php/ribosomal_antibiotics]

Production of antibiotics

Many microorganisms, including different species of fungi and bacteria, naturally produce antibiotics as secondary metabolites. (8) Penicillin, one of the most well-known antibiotics, is a secondary metabolite of the fungi *Penicillium*. Industrially used fungi strains for the production of penicillins (e.g. P. chrysogenum) secrete their products into the fermentation medium. This medium is then filtered and the penicillin is extracted. (9)

Biosynthesis of penicillin

Penicillin chemically belong to the group of beta-lactam antibiotics. They are produced from the amino acids L-a-aminoadipic acid (L-a-AAA), L-cysteine and L-valine (see **Figure 3**). The first step of the synthesis pathway is the formation of the tripeptide δ-(L-aaminoadipyl)-L-cysteine-D-valine (ACV), which is catalysed by the enzyme δ-(L-aaminoadipyl)-L-cysteine-D-valine synehetase. As a second step, isopencillin (IPN) N is formed by oxidative ring closure of the linear tripeptide by the enzyme isopenicillin N synthase. IPN possess weak antibiotic activity. Third, the hydripholic L -a-AAA side chain of IPN is exchanged for a hydrophobic acyl group. This reaction is catalysed by isopenicillin N acyltransferase (IAT). (10)

Semi-synthetic penicillin

Semi-synthetic penicillins are produced from an intermediate product of the biological production of penicillin. They are produced from intermediate products including 6-APA and 7-ACA, which are produced by removal of the acyl side chain from the purified penicillin produced by fungi. These acyl side chains are then produced by various groups to produce penicillin variants with different antibiotic resistances. (9)

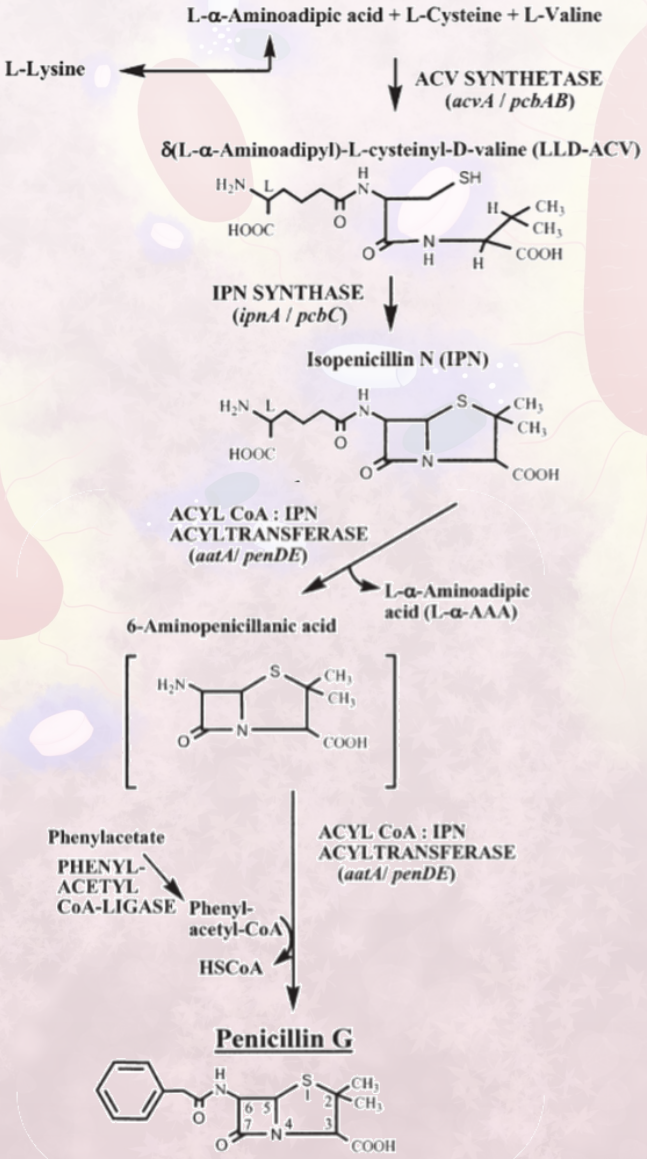


Figure 3. Biosynthesis of Penicillin. Adapted from Brakhage et al. 2004. (10)

Rise in antibiotic resistance

Antibiotic resistance is characterized by bacteria that have developed resistance to the antibiotics that were designed to kill them. As a result, certain antibiotics become ineffective, and the resulting infection becomes much more serious. In many cases, antibiotic-resistant infections require hospitalization and the administration of expensive and potentially toxic alternative treatments. In the US alone, 2 million people acquire an antibiotic-resistant infection each year, of which 23 000 cases are deadly. Antibiotic resistance has been recognized since the introduction of the first antibiotic, penicillin, in 1928. (11) Since then, it has become a growing problem, with emerging bacteria that can transmit their resistance genes to other bacterial species, spreading the resistance at an incredibly fast rate.

How does antibiotic resistance develop?

During a bacterial infection, some bacteria are present in your body that have developed resistance genes. These genes provide the bacteria with mechanisms that are able to prevent death by the antibiotic, for example by breaking down the antibiotic. If you treat an infection with a certain antibiotic, all but the resistant bacteria will be killed. Without the bacteria competing for nutrients and space, the resistant bacteria then have the opportunity to divide and grow, and cause infections that cannot be treated with that particular antibiotic anymore. (12)

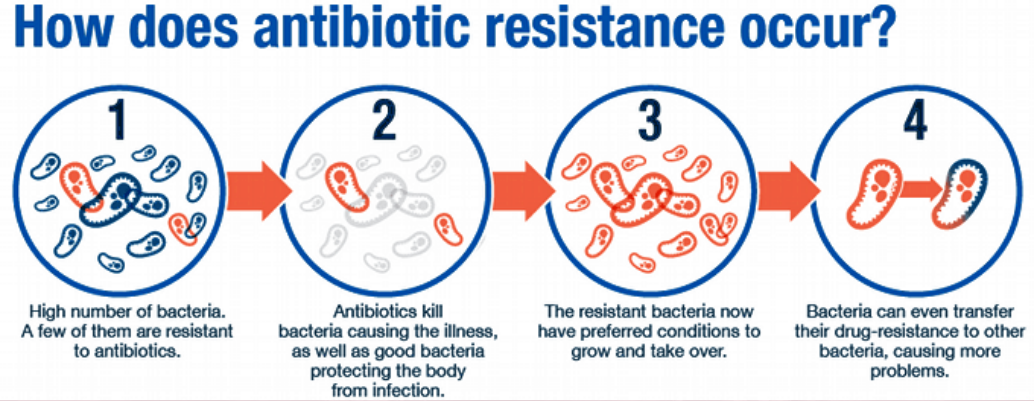
Which strategies do bacteria use to become resistant to antibiotics?

Bacteria have developed several tactics to become resistant to antibiotics. The first method is that bacteria can restrict access of antibiotics by limiting the number or size of pores in the cell wall through which the antibiotics usually enter. If they cannot prevent the antibiotic from entering the cell, they can develop mechanisms to simply pump the antibiotic back out of the cell, such as how the Pseudomonas aeruginosa gets rid of several antibiotic classes using specific pumps on its cell wall.
A third way of developing antibiotic resistance is the production of enzymes that degrade or chemically alter the drug, so it becomes ineffective. Another efficient strategy involves bypassing the effects of the antibiotics. For example, if the antibiotic disrupts the synthesis of important nutrients, the bacteria can develop a new pathway to produce the nutrient, thus making the antibiotic ineffective. Finally, modifying the structure of the antibiotic target can prevent it from being recognized and destroyed by the drug, allowing the bacteria to become resistant.

Why do antibiotic resistant bacteria cause a threat to public health?

Antibiotic resistance is considered one of the largest threats to global health. Although resistance to antibiotics is a process that occurs naturally and cannot be avoided, misuse of the antibiotics both in the clinic and in livestock has greatly accelerated the development of resistant bacteria. With increased global mobility of humans, antibiotic resistant bacteria spread easily, causing the number of resistant infections across the globe to rise. This way, infections that used to be easily treatable with first-line antibiotics, such as tuberculosis, pneumonia, sepsis and salmonellosis, turn into challenging infections that require the use of more expensive antibiotics, prolonged hospital stays, and increased mortality. Drug-resistant tuberculosis kills 250 000 people every year. Additionally, resistant infections arising as complications from surgeries or opportunistic infections of immunocompromised people, such as patients receiving chemotherapy, pose a serious risk for the otherwise life-saving medical advances. (13)

An analysis conducted by the WHO in 2018 involving 500, 000 patients with infections across 22 countries found that the most commonly encountered resistant bacteria are Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, and Streptococcus pneumoniae, followed by Salmonella species. Up to 82% of the reported infections were antibiotic resistant. This report shows the impact that the increasing incidence of antibiotic resistant bacteria has on global health. Existing treatments that are able to effectively treat these common infections lose their potency, posing a great risk of mortality to the affected patients. (14)



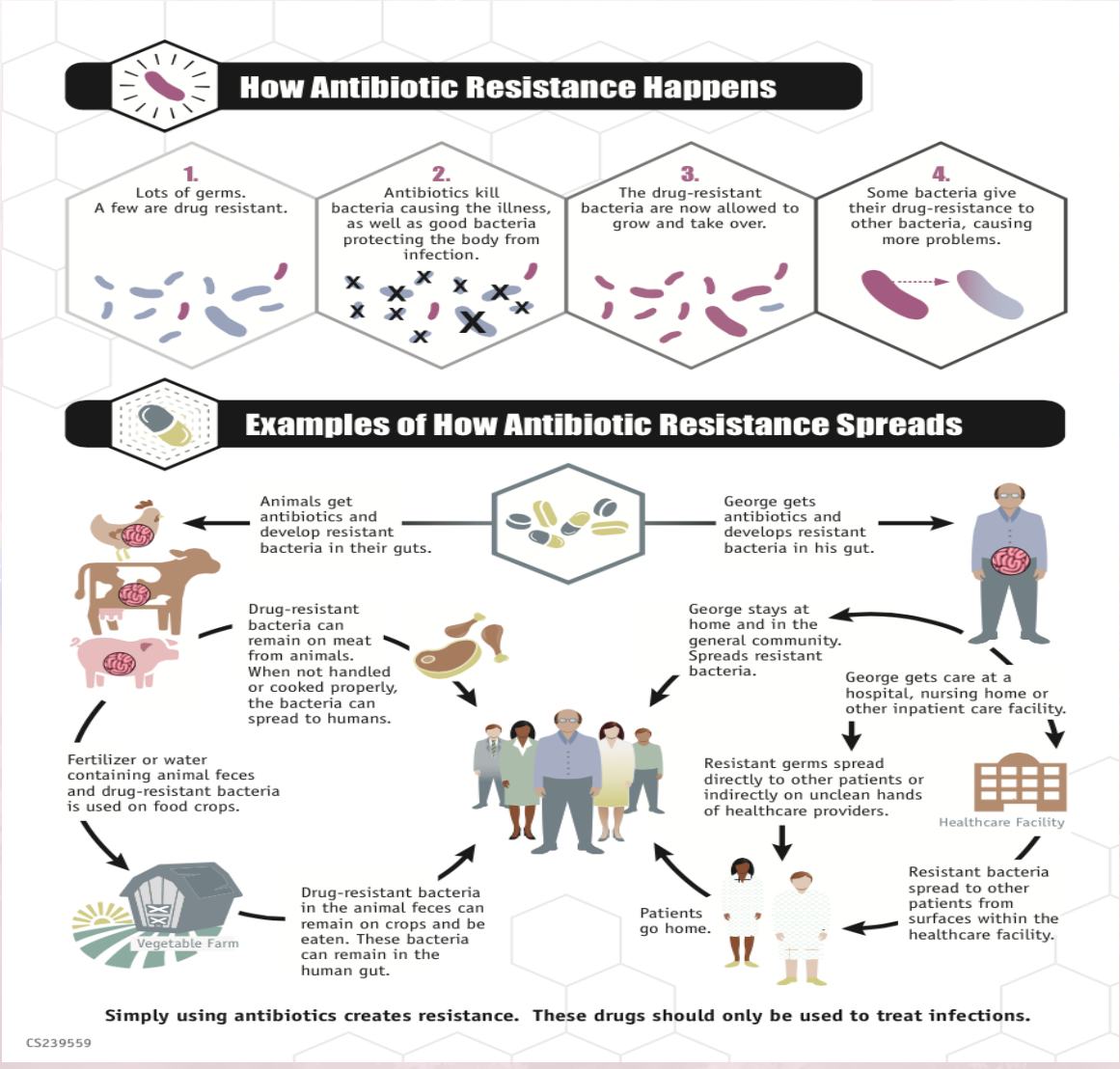
Furthermore, a report of the WHO in 2017 has found that the world is running out of antibiotics. There are too little antibiotics in development to tackle the emerging resistances. Although they identified 51 antibiotics or biologicals under development, only 8 of them are considered to be valuable candidates for the treatment of resistant infections. Especially the lack of new oral antibiotics is a serious issue, as it limits the possibility to treat these infections to hospital settings and areas that have higher resources.

What is being done to combat antibiotic resistance?

There is not enough research being done in the development of new antibiotics. In order to tackle this problem, the WHO and the Drugs for Neglected Diseases Initiative (DNDi) founded the Global Antibiotic Research and Development Partnership (known as GARDP). In September 2017, Germany, Luxembourg, the Netherlands, South Africa, Switzerland, the United Kingdom of Great Britain and Northern Ireland, and the Wellcome Trust invested more than €56 million for this work. (14)

Many countries have since joined forces, passed legislations, and set up funds to support the fight against bacterial resistance. Surveillance systems allow tracking of the occurrence of infections and the use of antimicrobial drugs. Furthermore, the requirement of prescriptions for antibiotic use in humans greatly reduces the amount of antibiotics that are over- and misused. Preventing the drugs from entering the environment, where they can cause resistant bacteria to develop and spread, is also an important strategy. Filters in wastewater plants can prevent the drug molecules to enter drinkable water, as antibiotics in drinking water are consumed by humans and give rise to resistant bacteria.

More and more countries restrict the use of antibiotics that are crucial to treat human infections in livestock. However, the greatest contribution comes from countries with high incomes, while developing countries do not have the resources to pass new legislations and set up surveillance methods. Therefore, it is crucial that the high-income countries support the developing countries in following guidelines and installing surveillance mechanisms. (15)



New and upcoming antibiotics

In one of the previous articles in this issue, it was explained that microbial resistance to antibiotics is a large threat to global health. A report of the American Centre of Disease Control and Prevention in 2013 stated that 23,000 patients die yearly due to antibiotic-resistant infections (16), which clearly shows the rising demand for new antibiotics. However, current antibiotic development has slowed down immensely. 20 new antibiotic classes were previously discovered and developed between 1940-1962, with only two new major classes being approved and commercialized since (17). This is mainly caused by large pharmaceutical companies shifting their focus to more profitable areas, such as treatments to lifestyle diseases (18). Although, smaller companies and academic scientists have made some progress in the field of antimicrobial therapy, leading to potential new targets and strategies in the war against bacteria and resistance (19).

New antibiotic targets

Traditionally, antibiotics have targeted bacterial DNA replication, protein synthesis, or peptidoglycan synthesis. One of the latest targets that has been under investigation is the biosynthesis of essential amino acids, which are amino acids that can only be synthesised by bacteria (17). An example of such an antibiotic is an inhibitor of the L,L-diaminopimelate aminotransferase (DapL) pathway of lysine synthesis, which is used in approximately 13% of known bacteria. Hence, research into DapL inhibitors is still in the early phases of screening for a lead compound (19).

In addition to amino acid biosynthesis, bacterial lipid biosynthesis also presents a potential target (17). A new promising antibiotic that targets lipid synthesis is teixobactin. It inhibits cell wall synthesis by binding to a highly conserved lipid II and III motif in Gram-positive bacteria. Since the structure of these lipids is not likely to change via mutations, resistance is not expected to develop (20).

Host lipid flows is seen as another possible target. Lipids are very important for bacteria during infection. They use and manipulate lipid signalling cascades, and use cholesterol to build neo-organelles and enter cells. However, bacteria are unable to synthesize cholesterol and some other lipids themselves. Therefore, inhibition of lipid synthesis or distribution in the host may inhibit bacterial growth (21).

Lastly, a target that has recently gained interest is quorum sensing, a molecular mechanism used by bacteria to communicate and subsequently adapt their behaviour to the cell density and environment (22). Quorum sensing will eventually lead to the secretion of virulence factors and the formation of biofilms. This makes quorum sensing an attractive target, as biofilms have long been associated with resistance to antibiotics (23). The potential of quorum sensing inhibitors (QSIs) was proven by animal studies of mutations in quorum sensing-related genes, which showed a reduced infection severity (24,25). Aside from their antimicrobial activity, QSIs are also able to restore antibiotic susceptibility in biofilm infection and enhance antibiotic efficacy when co-administered with said antibiotic (25).

New antimicrobial strategies

Alternative strategies to small molecule antibiotics are currently being investigated. A very promising strategy is the use of bacteriophages, which are viruses that are capable of infecting and killing bacteria (**Figure 4**). They work by binding to specific receptors on the cell surface, and inject their genomes inside the host cell. The viral genome is then copied, leading to the production of viral proteins used to form new virus particles. These then cause cell lysis and the release of the new particles. Bacteriophages have several advantages over traditional antibiotics. Firstly, they can be used to specifically attack antibiotic-resistant bacteria, and prevent the spread of resistant bacteria. It has also been speculated that the bacteriophages might eliminate them. Secondly, they are able to target pathogenic bacteria due to their specificity, leaving any beneficial bacteria unharmed. This is especially advantageous for the gut microbiota. Finally, resistance to bacteriophages is expected to be less of a problem, because, when the bacteria evolve to become resistant, the bacteriophages are able evolve to be able to attack the resistant bacteria (26). Additionally, several studies have shown a reduced virulence in pathogenic bacteria after mutations that led to phage-resistance (27,28). While monotherapy with bacteriophages is already quite effective, co-administration with sub-lethal doses of antibiotics has been shown to increase this effect (29).

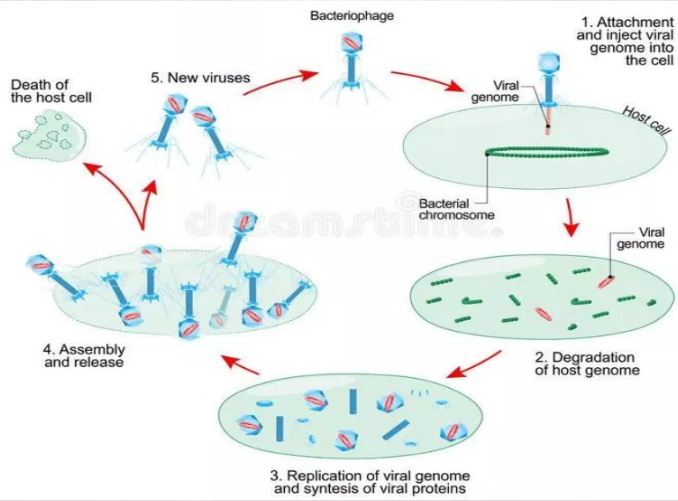
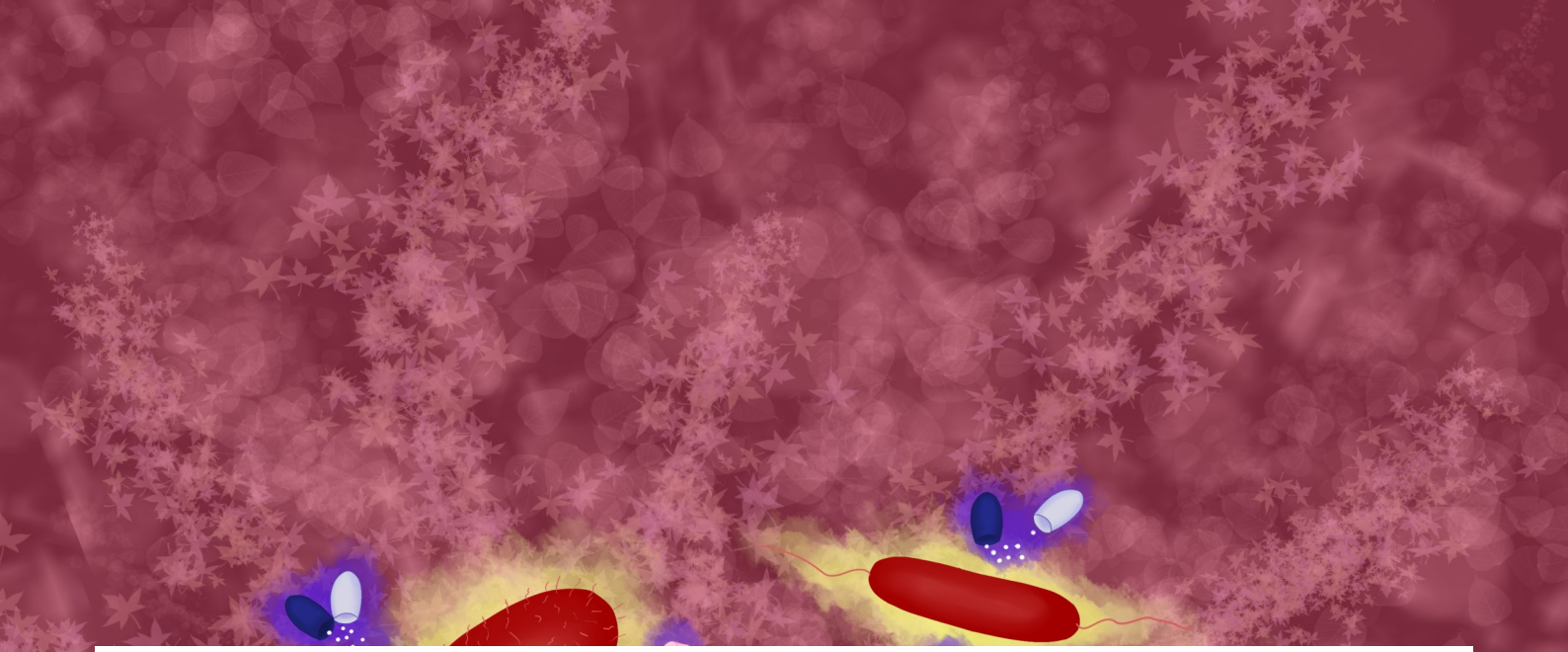


Figure 4. *The life-cycle and replication of bacteriophages.*



Another new strategy that synergistically enhances the antibiotic effect is the use of magnetic nanoparticles (MNP) as drug carriers. MNPs can be guided through the body using an external magnetic field, so lower doses can be administered (30). The use of MNPs can also reduce side-effects and increase efficacy. This increase can be explained by the intrinsic antimicrobial activity of the particles themselves, which is carried out by either damaging the cell wall, releasing toxic metals, or generating reactive oxygen species (30,31). The combination of the antibiotic efficacy and the MNP efficacy generates the synergistic effect. However, the drawback of this strategy is the limited amount of knowledge available on the toxicology and clearance of MNPs (30).

An emerging area in the field of antimicrobial therapy is the use of therapeutic antibodies against bacteria. The key advantages of antibodies over antibiotics are their specificity and their preventative use for infections. (17). There is currently only one antimicrobial antibody on the market called palivizumab, which is used against respiratory syncytial viral (RSV) infection in neonates. Other promising antibodies are in advanced stages of development (32).

Even though antibiotic development has been slow in the past 50 years, progress has still been made. Many significant discoveries were done, which have lead to the investigation and development of many promising new targets and strategies. These all have potential and advantages over the classical antibiotics. The use of bacteriophages especially seems to be a very promising method in possibly eliminating resistant bacteria. In conclusion, the solution to the problem of antibiotic resistance is being sought for, and might be closer than we think.

Nobel Prize Winner Ben Feringa

Ben Feringa is a Dutch chemist who studied chemistry at the University of Groningen, where he also did his PhD research on asymmetric oxidations of phenols and was appointed full professor in 1988. He combined his research and teaching after his study, and became a member of different academies in Europe and the USA. Over the years, his research has been awarded with several prices, including the Chirality medal (2009), the Marie Curie medal (2013), and the Nobel Prize in Chemistry (2016). Moreover, his research group on synthetic organic chemistry focuses on molecular nanoscience, synthesis and catalysis, and on biohybrid systems. For his work on molecular motors, he won the Nobel Prize. (33)

One of his most recently published articles is about how to use light to control antibacterial activity. (34) Due to severe side effects of drugs on humans and the environment, it is important to investigate ways to decrease these unwanted effects. This is especially necessary for antibiotics because of rising resistance. Antibiotic resistance is a huge threat and could kill millions of people eventually, according to Feringa. (35) Photoisomerization is one strategy currently being developed/used to tackle these issues. By shining light at a specific location in the body, the drug is activated. In this way, the drug acts more locally, which reduces the systemic side effects. At the moment, this technique is limited to the use of UV light, which has some disadvantages, as it is toxic to healthy cells, incapable of penetrating body tissues, and can activate the drug before it is administered. Therefore, Ben Feringa and others investigated if visible light can be used instead.

They investigated this by adding azobenzene, a UV light responsive photoswitch, to trimethoprim, an antibiotic chosen because it is frequently used and resistance against it is a growing problem. By changing the structure of the switch, which was eventually chosen to be a tetrafluoro-substituted azobenzene, its characteristics were optimized. The molecule was turned into the active cis-isomer with the use of green light (527 nm). An advantage of this switch is that the molecule can also be turned off, where radiation with violet light (400 nm) causes the molecule to switch back to the inactive trans-isomer. Although, due to the disadvantages of using UV light, they tried to edit the switch in such a way that the molecule can be switched with red light instead. For this purpose, they changed the azenobenzene to tetra-ortho-chloroazabenzene (**Figure 5**). Unfortunately, the photoisomerisation of this molecule was inefficient, and was therefore not tested in vitro with the presence of bacteria. Nevertheless, it was the first time an antibacterial agent was ever switched using light over 650 nm. (34)

In an interview, Ben Feringa said that the research is still fundamental. He also mentioned that the research into antibiotics does not just involve chemistry. Chemistry is the basis and other fields, such as biology and medicine, are also needed to solve the issue. For good research, you need multidisciplinary teams. It can take a few more years before concrete applications will appear. (35) In addition, despite the inefficient photoisomerisation with red light, this approach is a promising new resource in the face of the growing problem of antibiotic resistance.

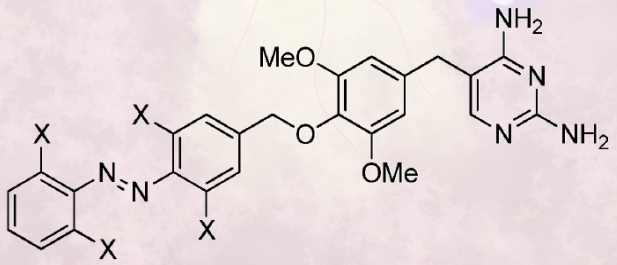


Figure 5. The structure of tetra-ortho-chloroazabenzene (left side of the molecule) coupled to trimethoprim (right side of the molecule). The X represents chlorine. (34)

Gut microbiota

You might not see them, but in and on every individual are billions and billions of microorganisms. Your intestines alone have a mutualistic symbiosis with about 10^14 of them, which corresponds to around 1 kilogram of microbiota (36). With gut microbiota, we mean all the bacteria, archaea, and eukarya that colonise the gastrointestinal (GI) tract. Besides helping the body digest and process food, there is accumulating evidence that these microorganisms play a significant role in health and disease. Several alterations in the normal composition and number of the gut microbiota have been implicated in different diseases, amongst which are; Autism Spectrum Disorder (ASD), Crohn's disease (CD) and Inflammatory Bowel Disease (IBD), obesity, and Parkinson's disease (PD). Even though the gut microbiota consists of more than just bacteria, most studies have focused primarily on researching those specific components of the microbes. There is also a lot less known about the fungal microorganisms, which the host body has a mutualistic agreement as well.

Development of microbiota

In the past, it has always been considered a dogma that the development of the host microbiota starts after birth. However, several studies suggest otherwise, as the presence of some microbes was noticed in the placenta during pregnancy (37). The exact development of the composition of the gut microbiota depends on numerous factors, such as the diet of the mother during pregnancy, the method of delivery, genetic components, early feeding patterns, and geographical location (36-38). After birth, the GI tract is rapidly colonized by types of bacteria, fungi, and archaea. During the first year of life, the microbial diversity develops quickly. After a child reaches the age of two and a half, the gut microbiota closely resembles one found in adults. In individuals of 65 and older, the abundance of several bacterial phyla increases, whilst in centenarians, the diversity of microbiota is significantly reduced. This shows that over the course of a lifetime, the gut microbiota may alter a lot based on environmental factors, such as living area (rural or urban), changes in diet, smoking, depression, surgery, or antibiotic treatment (37).

Normal composition

There is no 'normal' composition of the gut microbiota when we compare all individuals. Though, the most comprehensive view of the bacterial composition of the human body has been provided by combining data from the MetaHit and the Human Microbiome Project (37). There seems to be somewhat standard ratios of bacterial phyla in the GI tract, as well as core-functions that are upheld by the bacteria. Despite this, no individual has the exact same species or number of microbes in their intestines as another. Generally, the GI tract is seen as a system where two phyla are the most important (38), namely Firmicutes and Bacteroidetes. Here, the Firmicutes comprise gram-positive cell wall bacteria, whereas Bacteroidetes are composed of gram-negative bacteria.

Not all bacteria occur at the same spot in the GI tract. The density and composition of the microbiota are affected by several factors, including chemical, nutritional, and immunological gradients along the gut (37). For example, the small intestine is an environment with high levels of acids, oxygen, and antimicrobials, which are all limiting for the growth of bacteria. The result is that only fast-growing, facultative anaerobes are present here, as they have the ability to adhere to epithelia or mucus, and are therefore able to survive. Also, the faecal, luminal, and mucosal compositions differ. Whilst the abundance of *Bacteroidetes* is higher in the lumina, the *Firmicutes*, amongst which the species *Clostridium cluster XIVa,* are especially enriched in the mucus layer.

Function of the bacteria that benefit the host

Through millions of years of evolution, the microorganisms in our GI tract have proven themselves very useful in a number of ways. Their core activity is carbohydrate fermentation, with which they drive the energy and carbon economy of the colon (38). In fact, 10% of our daily energy requirement is provided by the bacteria in the colon through this type of fermentation. Even the metabolites called Short Chain Fatty Acids (SCFAs) that are formed during fermentation, such as butyrate, propionate, and acetate, play key roles in cell signalling and immune regulation in the periphery and fatty tissue.

Of these three SCFAs, butyrate is the most important one in terms of human health, as it has potential anti-colorectal -cancer activity by inducing apoptosis in colon cancer cells, or by regulating the expression of genes by reducing the activity of histone deacetylases. It is mostly produced by Firmicutes, whereas propionate is primarily produced by Bacteroidetes. Propionate is an energy source for epithelial cells, and it exerts a function in the liver in gluconeogenesis, with which it indirectly reduces obesity. Acetate, which is produced by many cell types, is an essential cofactor for the growth of other bacteria. Some bacteria will not even grow in pure culture when acetate is not present (39).

Besides this, the microorganisms in your gut are also responsible for absorption of nutrients, production of vitamins, amino acids synthesis out of ammonia and urea, stimulation and development of the host immunity, detoxification of xenobiotics, prevention of overgrowth or infection with pathogenic bacteria, and keeping our colonic wall healthy (40). All in all, the ecosystem in our intestines is of incredible value to our health.

Microbiota in disease

Not only is the presence of these microbes in our GI tract important, but also their exact composition in the body is crucial for our health. Once certain species or phyla increase or decrease in abundance, dysbiosis may occur, which can lead to disease processes. A specific example of this is IBD or CD. Evidence has been found that in both cases, the most consistent change is the reduced amount of *Firmicutes* and the increase in *Bacteroidetes* abundance, in comparison to healthy individuals. An association between the intestinal dysbiosis and obesity has also been found when this occurs the other way around. In obese mice, the abundance in *Bacteroidetes* is decreased, combined with a proportional increase in *Firmicutes* (38). This was confirmed in humans when obese individuals experienced weight loss after a dietary intervention that led to an increase in the relative abundance of *Bacteroidetes* (41).

A connection between alterations in the gut microbiota composition and neurological disorders has also been investigated in-depth. It is believed that through a gut-brain axis, the microbial composition has an influence on the mental health of an individual. For instance, through peptides that are sent to the gut once the body is satiated, the CNS causes a change in nutrient availability in the intestines. The other way around, the microbes in the GI tract manage to control several CNS activities via neural, endocrine, immune, and metabolic mechanisms (40). For example, a *Campylobacter jejuni* infection in the intestine has been shown to increase anxiety behaviour in mice, presumably through a mechanism in which a bacterial signal is carried by vagal sensory neurons (42).

In ASD, a strong correlation between GI dysfunction and autism severity has been found. However, whether there is an actual cause-effect relationship remains to be proven (40). As for PD, GI problems are seen as a non-motor symptom of the disease often occurs years before the onset of motor symptoms (43). When comparing faecal samples of PD patients to healthy individuals, it was found that the abundance of *Prevotellaceae* was reduced significantly, and the relative abundance of the *Enterobacteriaceae* was positively related to the severity of several motor symptoms. This suggests that the intestinal dysbiosis in PD is related to the motor phenotype (43). Overall, microbial dysbiosis occurs in many diseases, and has been studied quite extensively. Although, whether the dysbiosis is a cause or an effect of the disease still needs to be determined (40).

Alterations of the human gut microbiota

It is now clear that changes in the human gut microbial composition are strongly correlated with numerous types of diseases. Yet, the question of how these changes come about remains. There are several factors that may induce dysbiosis, the most common ones being (a change in) diet, use of antibiotics, surgery, and smoking (37). The early feeding pattern of infants may be important in autism, because children who are breast-fed for at least 6 months have a lower risk of developing ASD (36).

Antibiotic treatment is also a big factor in changing the composition of the gut microbiota. Clindamycin, clarithromycin, metronidazole, and ciprofloxacin alter the microbiota structure for a certain amount of time depending on the individual. Treatment with ampicillin, sulbactam, and cefazolin affects not only the microbial ecology in the gut, but also the production of metabolites, such as acetyl-CoA and acetyl phosphate. These metabolites play key roles in several cellular processes, and alteration of the formation of these metabolites may be a pathway in clinical disorders (37). Besides that, treatment with antibiotics during pregnancy is a potential risk factor for infantile autism (36), whereas antibiotic treatment in adults may lead to the development of autoimmune diseases (44).

When taking everything together, it is beyond dispute that gut microbiota play a big role in our health and disease. The exact mechanisms behind this may not be known, but understanding how the microbial environment in our body works may lead to potential treatments for numerous kinds of diseases.

Green Chemistry project abstract

The ‘Green Chem project’ is an HPPS-project about the reduction of chemical waste from surface water. The project was founded in 2018, and is currently performed by six Pharmacy and CPS students: M. Bek, L. Brandenburg, J. van Eijndhoven, K. Hooijschuur, R. de Kock, and A. Prodan.

Chemical waste in surface water has become a big problem over the past decades. The use and production of medicines has increased tremendously, however, the purification process in sewage treatment plants is not designed to purify these high concentrations of chemicals. At this moment, only 60-70% of these chemicals can be efficiently removed from the water, but it is estimated that this percentage will drop even further over the next decade. At this point, the sewage effluent in the Netherlands contains 14-30 µg of pharmaceuticals per liter. This poses not only a threat to the contamination of drinking water, but also has devastating effects on aquatic life. Yet, no proper regulation of pharmaceuticals in waste water has been implemented. The need for new legislation and “greener chemistry” is therefore of utmost importance. The ‘Green Chem project’ investigates the problems and possible solutions by conducting extensive literature research. New methods for the removal of pharmaceuticals from waste water are investigated, as well as “greener” chemical reactions to minimize the waste production and side product formation. Next to this, meetings with experts are organized to discuss this issue. For example, the water treatment plant Hoogheemraadschap De Stichtse Rijnlanden and the KWR are visited. Practical experiments, such as the identification of pharmaceuticals in water and new catalytic reactions are also part of the ‘Green Chem project’. Not only will the progress of this project be presented at the end-of-the-year HPPS meeting, but also a discussion by waste removal expert Lucas Schoenmakers will be included in this meeting.

Internship abstracts

Bacterial TLR5 inhibitor

Laurence Cleenewerk

I am currently finishing up my 5 month internship in the department of Infectious Diseases and Immunology, at the faculty of veterinary medicine, where I worked on identifying a small molecule TLR5 inhibitor secreted by a specific species of bacteria that lives in our gut.

The human GI tract is home to an estimation of over 10^14 microorganisms, including bacteria, archaea and eukaryotes, many of which have not yet been identified. (1) An imbalance of "good" and "bad" bacteria, or dysbiosis, contributes to the development and pathophysiology of diseases, such as autism, diabetes, and IBD. The gut microbiota communicates with the human host by secreting a large amount of different molecules. Some of these molecules can either promote or inhibit an inflammatory reaction. Inflammation is mediated through signaling induced by pattern recognition receptors (PRRs) that recognize bacterial compounds. One important class of PRRs are Toll-like receptors (TLRs). TLR5 is expressed on innate immune cells and gut epithelial cells, and uniquely recognizes the bacterial protein flagellin. Flagellin aids in invasion of the gut epithelial cells, and is an important target to recognize invading, pathogenic bacteria. Some bacteria, however, have modified amino acid sequences in their flagellin to avoid TLR5 recognition, which allows them to invade the epithelium and cause disease. So far, this is the only identified TLR5 evasion strategy employed by bacteria. I have discovered a soluble small molecule inhibitor of TLR5-mediated inflammation, secreted by a specific species of bacteria that usually live in our gut without causing disease. Identifying this molecule and its working mechanism could provide new strategies for treating bacterial infections by supporting the natural immune defense against invading bacteria. Furthermore its inhibitory function could be useful in diseases characterized by a leaky gut, where inflammation is exacerbated by exposure of TLR5 to high amounts of commensal flagellin, such as IBD.

Psychological fatigue in spondyloarthritis

Marije Voskamp

Spondyloarthritis (SpA) covers several disorders characterized by inflammation of the joints of the vertebral column that affects many people each year. SpA is closely associated with the MHC class I surface antigen subtype HLA-B27. Besides the physical symptoms, 75% of SpA patients report chronic psychological fatigue among their most severe symptoms. Even though it is widely accepted that chronic inflammation can cause depressive-like symptoms,including fatigue, no consensus has arisen concerning the association between inflammatory state and psychological fatigue in SpA. The mechanisms underlying inflammation-induced fatigue are not well understood and pain, rather than the inflammatory state, is often used as an indication for disease activity.

I performed my internship at the neuro-immunology group of Mechiel Korte, in the Department of Pharmacology. We hypothesized that psychological fatigue in SpA is dependent on disease activity in terms of inflammation and independent of pain. My internship consisted of an animal study, in which we performed behavioural tests indicative of psychological fatigue in HLA-B27 transgenic rats.

Furthermore, serum and brain samples will be analyzed to assess the association between inflammation in the brain and behavioural changes. Levels of certain neurotransmitters will be measured as an indication of indoleamine-2,3-dioxygenase (IDO) pathway. The IDO activity is increased during inflammation, causing increased synthesis of Quinolinic Acid, which promotes the downregulation of glutamate reuptake receptors (EAAT2) and the upregulation of glutamate release in astrocytes. This IDO pathway might induce depressive-like symptoms, including psychological fatigue and anhedonia.(45)

Investigating the function of a mucin receptor

Lamyae Chemlal

In our stomach and intestines exist important receptors that not many know about. These are called mucin receptors, and play an important role in innate immunity. Mucin receptors can be differentiated into membrane-bound mucins and secretory mucins, which are found are found in extracellular spaces. The membrane-bound mucin receptors can form a barrier that protects the gastrointestinal epithelial cells from invading pathogens. (1) Also, these mucins have shown to play a role in cell differentiation, proliferation, and regulating cellular signalling cascades. (2) The secretory mucins, on the other hand, are a major component of mucus, and can move around to trap debris. (3) This can be seen in Figure 6, where the microbiota is kept at a distance from the intestinal cells by mucins. (4)

Multiple studies have demonstrated direct or indirect interactions between membrane-bound mucins and other receptors, including toll-like receptors (TLR) and the epidermal growth factor receptor (EGFR). These mucins can then cause variations in immune activation, and thereby, inflammation. For instance, the transmembrane MUC1 receptor has shown to reduce the production of pro-inflammatory cytokines by suppressing TLR-induced NF-kB activation. The EGFR was also seen to be involved, as it phosphorylates the tail of the MUC1 receptor. (5) It is necessary to investigate such processes that may be used to study diseases where mucins are altered, such as IBD. Therefore, the aim of my research project was to study these interactions, in order to further understand its function in innate immunity and disease.

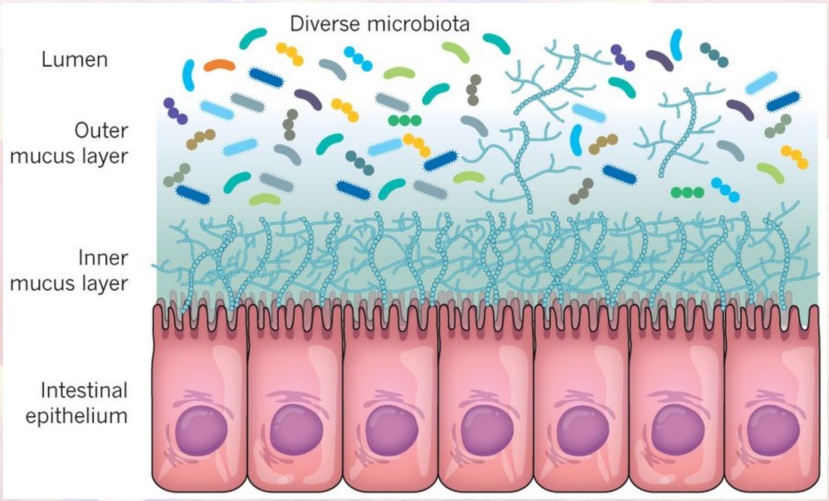


Figure 6. An illustration of mucins protecting the intestinal epithelial cells.

PhD abstracts

MUC1 is a receptor for the *Salmonella* SiiE adhesin that enables apical invasion into enterocytes

PhD(c): Xinyue Li

Earlier this year, Xinyue Li has published a major discovery in the field of Infection Biology and Immunology. She has studied the interaction between the foodborne bacteria *Salmonella enterica* and the MUC1 transmembrane mucin receptor, a highly glycosylated mucin receptor, in the gastrointestinal tract. Transmembrane mucin receptors that are expressed on epithelial cells can form a defensive barrier against pathogens, as a result of their glycosylated extracellular domain. (51) MUC1, in particular, is very heavily glycosylated, and extends far from the cell surface, as shown in **Figure 7**. (52)

In the case of *Salmonella,* invasion can occur from the apical surface where MUC1 is expressed, and, to a less extent, the lateral surface of epithelial cells. Previous research has shown that invasion occurs by *Salmonella* binding to a specific receptor using a giant SiiE adhesin. This is secreted by its type 3 secretion system, also used to inject virulence factors into cells to stimulate its uptake via macropinocytosis. Xinyue has further demonstrated that the specific receptor needed for apical invasion is the MUC1 receptor. She has established that the SiiE adhesin binds to sialic acids on the glycosylated extracellular domain of MUC1, making these acids necessary for apical invasion as well. Following this, large clusters of bacteria were found in the infected cells, while such clusters was not seen in lateral invasion. Moreover, since both the SiiE adhesin and the extracellular domain of MUC1 contain tandem repeats of DNA, it is hypothesized that binding is achieved using the tandem repeats, and that *Salmonella* can approach the cells in a zipper-like manner.

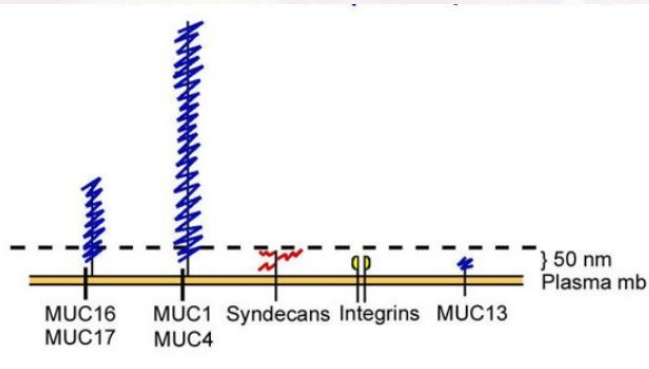


Figure 7. Comparison of transmembrane mucins and cell surface proteins. Adapted from (52).

All in all, this interesting discovery shows that transmembrane mucins can be used to attain invasion. Further investigations on this mechanism involved in such infections need to be conducted using other bacterial strains, potentially leading to new and better treatment options. (51)

Toll-like receptor biology: from evolution to function

PhD: Carlos Voogdt

On June 6th 2019, Carlos Voogdt successfully defended his PhD thesis with the title "Toll-like receptor biology: from evolution to function". For his achievements, he graduated *cum laude.* Carlos performed his PhD research at the faculty of veterinary medicine, in the department of Infectious Diseases and Immunology.

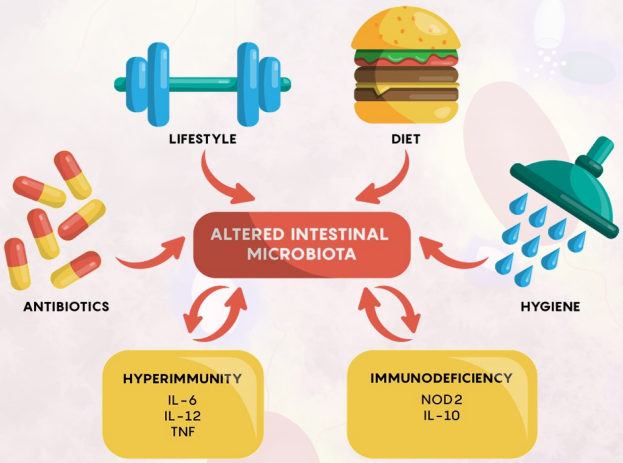
His research focuses on TLRs, an important class of receptors involved in the immune system. TLRs recognize a broad variety of molecules and induce signalling cascades upon activation. They recognize bacterial molecules and initiate mechanisms that allow clearance of pathogens, but they also respond to endogenous molecules, which has made them an interesting target in cancer and other immune disorders. Despite their crucial role in many diseases, their exact evolutionary function and response to ligands has not yet been fully understood. This leads to treatments targeting TLR functions being ineffective. In order to tackle this problem, Carlos investigated evolutionary changes of TLRs in different species. During evolution and the emergence of new species, changes in the structure of TLRs were necessary to adapt to new circumstances. By comparing the structure and function of TLRs in different species, such as crocodiles and chicken, specific features of TLRs that are important for a certain function become clear. However, one TLR that has remained highly conserved among species is TLR5. In his thesis, Carlos describes the first ever characterization of TLR5 in reptiles. TLR5 has previously only been identified in mammals and birds. Moreover, he showed that while TLR5 originating from both mammals and reptiles is able to recognize specifically the bacterial protein flagellin, the sensitivity towards flagellin of different bacterial species can differ between mammals and reptiles. For example, the response to *Pseudomonas* flagellin by a lizard-derived TLR5 was stronger than that of a human TLR5. Furthermore, he also identified the previously unknown function of the C-terminal tail region of human TLR5. Next to TLR5, Carlos also investigated the evolutionary function of another TLR, TLR15, found in birds and reptiles. (53)

Altogether, his work describes the importance of an evolution-based approach for identifying function of TLRs, and possibly other proteins. His work gives important insights into TLR function, which is relevant for the understanding of their role in human disease.

Latest news

Gut bacteria as a therapeutic target for food allergy

A recently published review by Canani et al. states that gut bacteria could be used as a target for future treatment for food allergy. This type of allergy is caused by a defective immune tolerance, which is, among others, influenced by gut microbiota function and structure. Associations between dysbiosis in the gut microbiota and food allergies were also made; therefore, the gut microbiota could be a new target for treating food allergy. There is also encouraging data from studies that have already been done on influencing the gut microbiome; although, more evidence is needed that can be translated in clinical practice. Nevertheless, influencing the gut bacteria, with either nutrients or probiotics, is promising for the future. (54)



Recovery of bacteria nearly killed by antibiotics

Nolivos et al. recently discovered a protein named the AcrAB-TolC multidrug efflux pump, that is responsible for the survival of bacteria that are nearly killed by antibiotics. The efflux pump cannot cause drug resistance on its own, but it helps the bacteria to become drug resistant. It does this by pumping enough antibiotic molecules out of the bacterial cell in order to allow production of real resistance proteins. Therefore, the bacteria can still become antibiotic resistant even though it was about to be eliminated. (55)

Mini kidneys produced from urine cells by Utrecht researchers

Schutgens et al. produced kidney organoids, called tubuloids (**Figure 8**), that can be established from human urine cells, and contain proximal and distal nephron segments. This research was a collaboration of scientists from Utrecht University, University Medical Center Utrecht, and Hubrecht Institute. The mini kidneys can be used to study diseases and also treatment options. It can be used to investigate which treatment works best for a viral infection, for example, that occurs with a kidney transplant. In the future, the researchers hope to be using the mini kidneys to create real functioning kidneys. (56)

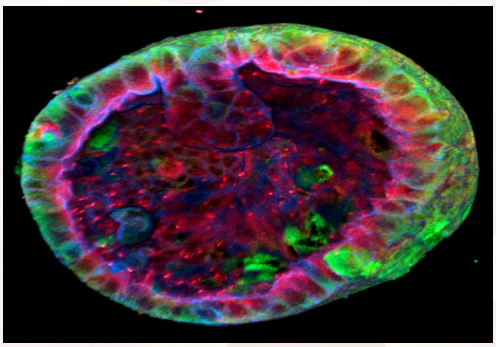


Figure. 8 A mini kidney known as tubuloid.

Dr. Jan van der Valk receives Björn Ekwall Memorial 2019

Dr. Jan van der Valk is director of the 3Rs-Centre Utrecht Life Sciences and partner in the Utrecht Advanced In Vitro Models Hub (U-AIM). He won the Björn Ekwall Memorial Foundation Award 2019, the first time a Dutch researcher has won this award. Van der Valk received the award because of his work on the awareness of the issue regarding the use of fetal calf serum (FCS). Animals are still indirectly used for in vitro research, because the FCS, a serum that is frequently used to grow cells, is harvested from living bovine foetuses taken from pregnant cows during slaughter. Therefore, the calves could be seen as experimental animals and many scientists are not aware of this. Van der Valk has tried to change this by giving lectures, organizing workshops, and publishing papers. (57)

AWARENESS CALENDAR

March - August 2019

March

- 26-03: purple day for epilepsy awareness
- Multiple sclerosis education and awareness month

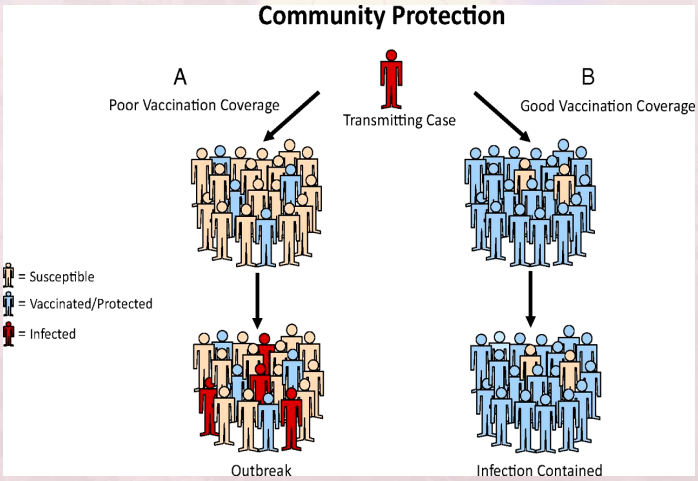
The 24th of March is World Tuberculosis Day. The Centers for Disease Control and Prevention (CDC), among other organisations, raise awareness to tuberculosis (TB). This year, the theme was 'It's TIME', meaning it is time to raise more awareness for the disease and to try to stop the disease for good. (58)

TB is the number one cause of death of infectious disease, caused by a bacteria, with 95% of these deaths occurring in developing countries. This is due to the inability of patients to access the vaccine against the *Mycobacterium tuberculosis* bacteria, which causes TB. Those with high risks, such as children and elderly are also unable to receive the vaccine, leading to further spread of the airborne disease. Moreover, approximately a quarter of the world's population is thought to be infected, but the disease is often latent, where it is neither symptomatic nor contagious. Nevertheless, latent TB can still develop into the symptomatic disease, and half of the infected patients can die, as the bacteria is often antibiotic resistant. (59)

April

- 07-04: world health day
- 24-04: worlds meningitis day

The last week of April (24th to the 30th) is the World Immunization Week. Immunization through vaccination is currently seen as one of the most cost-effective and successful interventions to prevent disease. The theme this year will be 'Protect Together: Vaccines Work!', and will be done by celebrating people who were very important in realizing vaccination and seen as 'vaccine heroes', such as parents or community workers. However successful vaccination may be, there are still many unvaccinated or under-vaccinated children, of which many still die from preventable diseases. In 2017, 116.2 million children were vaccinated; the highest amount of vaccinated children in history. Furthermore, huge strides were taken since 2010, as new vaccines were used in 113 different countries. Yet, not everyone is in favour of vaccination, even though research has proven their safety and efficacy. (60) It is, therefore, not only important to reach developing countries, but to keep focused on the developed world as well. It is also of great necessity that the vaccination minima are reached to provide herd immunity, which for some diseases is virtually 100%. (61)



May

- ◊ Mental health month
- ◊ Lupus awareness month

The 11th of May is Cornelia de Lange Syndrome (CdLS) Awareness Day. CdLS is a genetic disease which occurs in 1:10 000 to 1:30 000 people. The exact occurrence is not clear as the varying symptoms are often not recognized. Therefore, it is important to raise awareness for the disease, with the 11th of May being the day for this. The disease was first described in 1933 by the Dutch pediatrician Cornelia de Lange, who found two children with similar conditions. (62)

The disease occurs as a result of mutations in 5 genes that help regulate the cohesin complex, which is important in development before birth. Children born with this defect are often rather small with a short nose, longer and thicker eyebrows, and long lashes. The bones of the arms and hands do not develop normally due to the growth defect before and after birth, and the individuals often have mild to severe mental disabilities. During childhood, they often develop difficulties in social communication very similar to autism. People with CdLS mostly do not get children. However, if they only had mild symptoms they might, and those children often have CdLS as well. Nonetheless, the chance of people with a CdLS child of having another affected child are very slim (0.6%) if the parents do not seem to have the condition. (63)

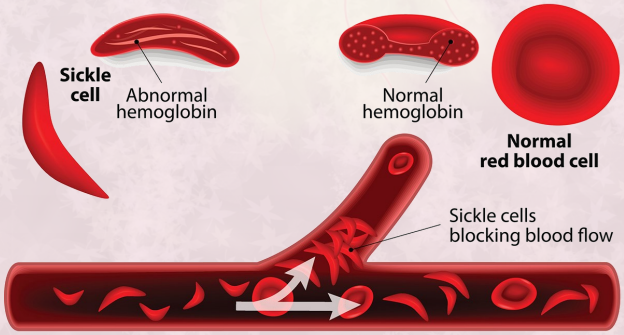
June

- ◊ 27-06: PTSD awareness day
- ◊ 23/29-06: Helen Keller Deaf-Blind awareness week

The 19th of June is World Sickle Cell Day. Sickle cell disease (SCD) is a very common inheritable blood disease. It is autosomal recessive disease, and is seen in approximately 75,000 Americans. (64) The disease is very common in regions such as Africa, the Mediterranean, and India. (65) Therefore, World Sickle Cell Day was organized in 2009 after the UN established it in 2008.

SCD influences the red blood cells through different hemoglobin proteins. Hemoglobin carries oxygen in the blood, and the complex normally consists of two alpha-hemoglobins and two beta-hemoglobins. In SCD, the beta-hemoglobins are different, causing the red blood cells to form half moons instead of their normal shape. As a result, the cells die more quickly and get stuck in small blood vessels, as they are less flexible.

Meanwhile, when only one copy of the SCD gene is carried, the person has capacities to fend off malaria, which is a disease caused by parasites in mosquitoes that leads to symptoms similar to the flu, but more severe with some forms being very deadly. This is due to the cell becoming a sickle upon infection and dying; therefore, protecting the individual from malaria as the virus cannot spread. On the other hand, people who have both copies of the faulty gene are highly susceptible to the disease, as it is thought that malaria makes the SCD worse through the same mechanism, as extra cells die. (66)



July

- 28-07: World Hepatitis Day
- International Group B Strep Throat awareness month

August

- 27-06: PTSD awareness day
- 23/29-06: Helen Keller Deaf-Blind awareness week

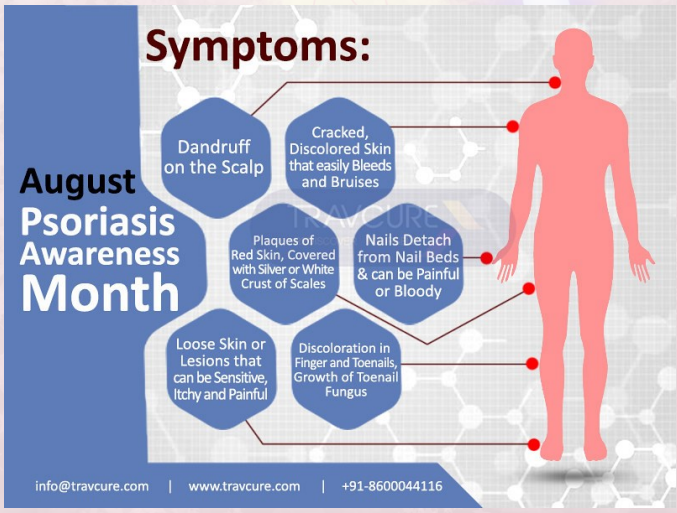
July is the month of Juvenile arthritis is not one disease, but a term that covers many autoimmune and inflammatory diseases in children under 16 years old. It is quite difficult to diagnose a child into the correct disease within juvenile arthritis, making it important that people are aware of the different diseases, medical history, and physical symptoms, along with a critical view of diagnostic devices. In addition, It has been established that a child only has juvenile arthritis if there is inflammation and pain for longer than six weeks. Children are often picky eaters, get ill easily after playing together, and fall down causing pain and stiffness. These are also all symptoms of juvenile arthritis, and they can occur at varying ages. (67)

There are many diseases included in juvenile arthritis, such as: rheumatoid arthritis, which is often seen in older people and mostly affects the joints; (systemic) lupus, which affects organs, such as the kidneys, and often causes skin rashes; fibromyalgia, which is seen more often in girls and is hard to diagnose; and Kawasaki disease, which causes inflammation in the blood vessels possibly leading to heart complications. Thereby, awareness of juvenile arthritis is important, as early diagnosis and treatment can prevent pain and permanent damage. (67-68)

Fleur van der Leij, 17 years old, was diagnosed with juvenile psoriatic arthritis in due to the continuing pain in her knees, elbow, and hands when she was 15 years old. Her joints were also swollen, and after a month she received the diagnosis and had to take ibuprofen three times a day and doxycycline for one month. We asked her about how she went through having this disease, and what she thought of the current awareness. She said her school was made aware, and after a final diagnosis, they did make sure she was taken care of when needed. Her doctors were fast in diagnosing her, but now, she still has an appointment every three months. She goes to the hospital to talk about the condition with others, and to raise awareness herself. She made the following comment on awareness: 'People do make adjustments for me if they know about my condition, like making sure there is a better chair, however most people do not know about the disease or what is happening to me'. With this, teaching others about the disease and spreading awareness is very important during this month, as well as others.

Psoriasis is a disease in which the skin rapidly regenerates, leaving behind red patches and scaly dead skin. The patches can burn, itch, or hurt, and can come and go or stay, even with medication. There are varying forms that differ in severity and how the skin rashes develop and look. It is thought to be triggered by T-cells that attack the skin cells and attract neutrophils. As T-cells are involved in fighting off pathogens and disease, the condition may worsen or start due to an infection, which causes activation of the T-cells or induce their response. The most severe form that covers the whole body is erythrodermic psoriasis, which is also the least common form. Even though the chronic disease is not contagious, people think it is scary, as the skin is distorted and can occur in the nails as well.

There is currently no cure for the condition, and the treatment available can only slow down the lifecycle of the cells. Still, lifestyle is an important factor in the development of psoriasis, as stress, smoking, or heavy alcohol consumption can affect the disease. People with psoriasis are more likely to develop varying diseases such as Parkinson's, emotional problems, kidney disease, type 2 diabetes, and multiple other autoimmune disorders. However, the cause for this correlation is still unknown. Aside from the awareness month, there is also an awareness day for Psoriasis, which is in the 29th of October.



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Next time...

This concludes this year's final issue! We hope that you have enjoyed reading it as much as we have enjoyed making it. This issue is very special to us, since it is the last one for some of us, including the founder: Laurence Cleeneweerk. It has been an honour making these educational and interesting issues for you all over the past year. We would like to thank all the members that have worked hard to make this journal, as well our supervisor Gert Folkers, the HPPS coordinator Dirk Rijkers, and our readers for motivating us all the time. This journal will continue throughout the next years, and will be brought to you by very enthusiastic members. We hope that you carry on following and supporting this journal, and wish you all a great summer vacation!

Also, don't forget to let us know if you would like to publish your project idea, thesis, abstract, project outcome, etc. You can contact us via our email: **hppsjournal@gmail.com**

See you next time!

The Drugs and Beyond Team